Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. (Withdrawn) A multicomponent vaccine comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide derived from a protozoan and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan.

2. (Withdrawn) The multicomponent vaccine of claim 1 wherein the protozoan is selected from the group consisting of *Trypanosoma, Leishmania, Toxoplasma, Eimeria, Neospora, Cyclospora* and *Cryptosporidia.*

3. (Withdrawn) The multicomponent vaccine of claim 2 wherein the protozoan is *T. cruzi.*

4. (Withdrawn) The multicomponent vaccine of claim 1 wherein the immunogenic polypeptide is at least one of a surface-associated or a secreted polypeptide.

5. (Withdrawn) The multicomponent vaccine of claim 4 wherein the immunogenic polypeptide is a GPI-anchored polypeptide.

6. (Withdrawn) The multicomponent vaccine of claim 1 wherein the immunogenic polypeptide is a member of the trans-sialidase family of proteins.

7. (Withdrawn) The multicomponent vaccine of claim 3 wherein the immunogenic polypeptide is expressed in a *T. cruzi* amastigote.

8. (Withdrawn) The multicomponent vaccine of claim 7 wherein the immunogenic polypeptide is selected from the group consisting of TSA-1, ASP-1, ASP-2, hemolysin and Lytl protein.

9. (Withdrawn) The multicomponent vaccine of claim 1 comprising at least ten immunogenic polypeptides derived from the protozoan or at least ten nucleotide coding regions encoding immunogenic polypeptides derived from the protozoan.

10. (Withdrawn) The multicomponent vaccine of claim 1 which stimulates at least one immune response in a mammalian host selected from the group consisting of an antibody response and a cell-mediated immune response.

11. (Withdrawn) The multicomponent vaccine of claim 10 which stimulates at least one of a Th1-biased $CD4^+$ T cell response or a $CD8^+$ T cell responses.

12. (Withdrawn) The multicomponent vaccine of claim 11 which stimulates a $CD8^+$ T cell response.

13. (Withdrawn) The multicomponent vaccine of claim 10 which stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response.

14. (Withdrawn) The multicomponent vaccine of claim 1 comprising a plurality of polynucleotides comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

15. (Withdrawn) The multicomponent vaccine of claim 14 wherein the cytokine is selected from the group consisting of interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating

factor (GM-CSF), interleukin-6 (IL-6), interleukin-18 (IL-18), γ-interferon, α,β-interferons and a chemokine.

16. (Withdrawn) The multicomponent vaccine of claim 1 comprising a plurality of immunogenic polypeptides derived from a protozoan, wherein the immunogenic polypeptide comprises a membrane translocating sequence.

17. (Withdrawn) The multicomponent vaccine of claim 16 wherein the membrane translocating sequence is derived from HIV TAT protein.

18. (Withdrawn) The multicomponent vaccine of claim 1 which is a therapeutic vaccine.

19. (Withdrawn) The multicomponent vaccine of claim 1 which is a prophylactic vaccine.

20. (Withdrawn) The multicomponent vaccine of claim 1 formulated for administration to a cat, a dog, or a human.

21. (Withdrawn) A vaccine comprising at least one component selected from the group consisting of (a) an immunogenic polypeptide derived from a protozoan and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan, wherein the vaccine stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response against the protozoan upon administration to a mammal.

22. (Withdrawn) The vaccine of claim 21 wherein the protozoan is selected from the group consisting of *Trypanosoma*, *Leishmania*, *Toxoplasma*, *Eimeria*, *Neospora*, *Cyclospora* and *Cryptosporidia*.

23. (Withdrawn) The vaccine of claim 22 wherein the protozoan is *T. cruzi*.

24. (Withdrawn) The vaccine of claim 21 wherein the immunogenic polypeptide is a surface-associated or a secreted polypeptide.

25. (Withdrawn) The vaccine of claim 23 wherein the immunogenic polypeptide is a GPI-anchored polypeptide.

26. (Withdrawn) The vaccine of claim 21 wherein the immunogenic polypeptide is a member of the trans-sialidase family of proteins.

27. (Withdrawn) The vaccine of claim 23 wherein the immunogenic polypeptide is expressed in a *T. cruzi* amastigote.

28. (Withdrawn) The vaccine of claim 23 wherein the immunogenic polypeptide is selected from the group consisting of TSA-1, ASP-1, ASP-2, hemolysin and Lyt1 protein.

29. (Withdrawn) The vaccine of claim 21 comprising at least one polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

30. (Withdrawn) The multicomponent vaccine of claim 29 wherein the cytokine is selected from the group consisting of interleukin-12 (IL-12), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-6 (IL-6), interleukin-18 (IL-18), γ-interferon, α,β-interferons and a chemokine.

31. (Withdrawn) The vaccine of claim 21 comprising at least one immunogenic polypeptide derived from a protozoan, wherein the immunogenic polypeptide comprises a membrane translocating sequence.

32. (Withdrawn) The vaccine of claim 31 wherein the membrane translocating sequence is derived from HIV TAT protein.

33. (Withdrawn) The vaccine of claim 21 which is a therapeutic vaccine.

34. (Withdrawn) The vaccine of claim 21 which is a prophylactic vaccine.

35. (Withdrawn) A pharmaceutical composition comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide derived from a protozoan and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan; and a pharmaceutically acceptable carrier.

36. (Withdrawn) A pharmaceutical composition comprising at least one component selected from the group consisting of (a) an immunogenic polypeptide derived from a protozoan and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide derived from a protozoan, wherein the immunogenic polypeptide or the polynucleotide stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response against the protozoan upon administration to a mammal; and a pharmaceutical acceptable carrier.

37. (Withdrawn) A recombinant method of making a multicomponent polynucleotide vaccine comprising:

(a) inserting a plurality of nucleotide coding regions encoding an immunogenic polypeptide derived from a protozoan into a plurality of polynucleotide vectors; and

(b) combining the polynucleotide vectors to yield a polynucleotide vaccine.

38. (Withdrawn) A recombinant method for making a multicomponent polypeptide vaccine comprising:

(a) providing a plurality of expression vectors comprising a nucleotide coding region encoding a membrane transducing sequence;

(b) inserting a nucleotide coding regions encoding an immunogenic polypeptide derived from a protozoan into each of the expression vectors in frame with the membrane transducing sequence to yield an expression vector comprising a nucleotide coding region encoding an immunogenic fusion protein comprising the membrane transducing sequence and the immunogenic polypeptide; and

(c) causing expression of the expression vectors to yield the immunogenic fusion proteins;

(d) purifying the immunogenic fusion proteins; and

(d) combining the isolated immunogenic fusion proteins to yield a polypeptide vaccine.

39. (Withdrawn) The method of claim 38 wherein purifying the immunogenic fusion proteins comprises destabilizing the fusion proteins with urea.

40. (Currently amended) A method for therapeutic immunization of a mammal harboring a persistent protozoan infection comprising administering to the infected mammal [[the]] a multicomponent vaccine ~~of claim 1~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to eliminate the parasite from the mammal.

41. (Original) The method of claim 40 wherein the protozoan is selected from the group consisting of *Trypanosoma*, *Leishmania*, *Toxoplasma*, *Eimeria*, *Neospora*, *Cyclospora* and *Cryptosporidia.*

42. (Original) The method of claim 41 wherein the protozoan is *T. cruzi*.

43. (Original) The method of claim 40 wherein the vaccine stimulates a $CD8^+$ T cell response.

44. (Currently Amended) The method of claim 40 wherein the multicomponent vaccine comprises a plurality of polynucleotides comprising a nucleotide coding region encoding an immunogenic polypeptide ~~derived from a protozoan~~ and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

45. (Currently Amended) The method of claim 40 wherein the multicomponent vaccine comprises a plurality of immunogenic polypeptides ~~derived from a protozoan~~, wherein the immunogenic polypeptide comprises a membrane translocating sequence.

46. (Currently Amended) A method for therapeutic immunization of mammal harboring a persistent protozoan infection comprising administering to the infected mammal [[the]] a multicomponent vaccine ~~of claim 1~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent or delay chronic debilitating disease in the mammal.

47. (Original) The method of claim 46 wherein the protozoan is selected from the group consisting of *Trypanosoma, Leishmania, Toxoplasma, Eimeria, Neospora, Cyclospora* and *Cryptosporidia.*

48. (Original) The method of claim 47 wherein the protozoan is *T. cruzi.*

49. (Original) The method of claim 46 wherein the vaccine stimulates a $CD8^+$ T cell response.

50. (Currently amended) The method of claim 46 wherein the multicomponent vaccine comprises a plurality of polynucleotides comprising a nucleotide coding region encoding an immunogenic polypeptide ~~derived from a protozoan~~ and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

51. (Currently amended) The method of claim 46 wherein the multicomponent vaccine comprises a plurality of immunogenic polypeptides ~~derived from a protozoan~~, wherein the immunogenic polypeptide comprises a membrane translocating sequence.

52. (Currently amended) A method for therapeutic immunization of a mammal harboring a persistent protozoan infection comprising administering to the infected mammal [[the]] a vaccine ~~of claim 21~~ comprising at least one component selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide, wherein the vaccine stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response against the protozoan upon administration to a mammal; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and, wherein administration of the vaccine is effective to eliminate the parasite from the mammal.

53. (Currently amended) A method for therapeutic immunization of mammal harboring a persistent protozoan infection comprising administering to the infected mammal [[the]] a vaccine ~~of claim 21~~ comprising at least one component selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide, wherein the vaccine stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response against the protozoan upon administration to a mammal; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent or delay chronic debilitating disease in the mammal.

54. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 1~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent subsequent infection of the mammal by the protozoan.

55. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 1~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide ; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan: wherein administration of the vaccine is effective to prevent the

development of chronic debilitating disease the mammal after subsequent infection by the protozoan.

56. (Original) The method of claim 55 wherein the protozoan is selected from the group consisting of *Trypanosoma, Leishmania, Toxoplasma, Eimeria, Neospora, Cyclospora* and *Cryptosporidia.*

57. (Original) The method of claim 56 wherein the protozoan is *T. cruzi.*

58. (Original) The method of claim 55 wherein the vaccine stimulates a $CD8^+$ T cell response.

59. (Currently amended) The method of claim 55 wherein the multicomponent vaccine comprises a plurality of polynucleotides comprising a nucleotide coding region encoding an immunogenic polypeptide ~~derived from a protozoan~~ and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

60. (Currently amended) The method of claim 55 wherein the multicomponent vaccine comprises a plurality of immunogenic polypeptides ~~derived from a protozoan~~, wherein the immunogenic polypeptide comprises a membrane translocating sequence.

61. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 1~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or

secreted by a protozoan: wherein administration of the vaccine is effective to prevent the death of the mammal after subsequent infection by the protozoan.

62. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 21~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent subsequent infection of the mammal by the protozoan.

63. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 21~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent the development of chronic debilitating disease the mammal after subsequent infection by the protozoan.

64. (Currently amended) A method for prophylactic immunization of a mammal against an infectious protozoan comprising administering to an uninfected mammal [[the]] a multicomponent vaccine ~~of claim 21~~ comprising a plurality of components selected from the group consisting of (a) an immunogenic polypeptide and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic polypeptide; wherein the immunogenic

polypeptide comprises a protozoan polypeptide that is associated with a protozoan cell surface or secreted by a protozoan; and wherein administration of the vaccine is effective to prevent the death of the mammal after subsequent infection by the protozoan

65. (Currently amended) A method for therapeutic immunization of a mammal harboring a persistent *T. cruzi* infection comprising administering to the infected mammal a multicomponent vaccine comprising a plurality of components selected from the group consisting of (a) an immunogenic *T. cruzi* polypeptide ~~derived from *T. cruzi*~~ and (b) a polynucleotide comprising a nucleotide coding region encoding an immunogenic *T. cruzi* polypeptide ~~derived from *T. cruzi*~~, wherein administration of the vaccine is effective to prevent or delay chronic debilitating disease in the mammal.

66. (Currently amended) The method of claim 65 wherein the multicomponent vaccine comprises a plurality of polynucleotides comprising a nucleotide coding region encoding an immunogenic *T. cruzi* polypeptide ~~derived *T. cruzi*~~ and at least one polynucleotide comprising a nucleotide coding region encoding a cytokine.

67. (Original) The method of claim 65 wherein administration of the multicomponent vaccine stimulates an antibody response, a Th1-biased $CD4^+$ T cell response and a $CD8^+$ T cell response in the mammal.

68. (Currently amended) The method of claim 65 wherein the multicomponent vaccine comprises a plurality of immunogenic *T. cruzi* polypeptides ~~derived from *T. cruzi*~~, and wherein the immunogenic polypeptide comprises a membrane translocating sequence.

69. (Original) The method of claim 65 wherein the mammal is a dog, a cat, or a human.

70. (Withdrawn) A method for identifying immunogenic protozoan polypeptides for use in a polynucleotide vaccine comprising:

(a) generating a protozoan genomic library; and

(b) employing the technique of expression library immunization (ELI) in mice to identify protozoan polypeptides that elicit an immune response in a mammal effective to prevent the death of the mammal or to arrest or delay the progression of disease in the mammal associated with infection of the mammal by the protozoan.

71. (Withdrawn) The method of claim 70 wherein step (a) comprises generating a *T. cruzi* genomic library.

72. (Withdrawn) A method for identifying immunogenic *T. cruzi* polypeptides for use in a polynucleotide vaccine comprising:

(a) preparing a DNA microarray comprising open reading frames of *T. cruzi* genes;

(b) preparing a first probe comprising Cy3-labeled trypomastigote-derived *T. cruzi* cDNA;

(c) preparing a second probe comprising Cy5-labeled amastigote-derived cDNA;

(d) cohybridizing the first and second probes to the microarray to identify at least one gene whose expression is upregulated in *T. cruzi* during the intracellular amastigote stage of the infectious cycle, which gene encodes a candidate immunogenic *T. cruzi* polypeptide; and

(e) immunizing mice with the gene to determine whether the gene encodes a *T. cruzi* polypeptide that elicits an immune response in a mammal effective to prevent the death of the mammal or to arrest or delay the progression of disease in the mammal associated with infection of the mammal by *T. cruzi*.

73. (Withdrawn) A method for treatment or prevention of a protozoan infection in a mammal comprising:

(a) administering to the mammal a polynucleotide vaccine comprising at least one of a plasmid DNA and a viral vector, the plasmid DNA and viral vector comprising at least one nucleotide coding region encoding an immunogenic polypeptide derived from the protozoan; followed by

(b) administering at least one of a polypeptide vaccine comprising an immunogenic polypeptide derived from the protozoan and a polynucleotide vaccine comprising a viral vector comprising a nucleotide coding region encoding an immunogenic polypeptide derived from the protozoan.